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DATA EVALUATION REPORT

Study Type: Evaluation of the Significance of Forestomach Tumors Induced in Rodents by CGA154281 (Benoxacor). Nonguideline

DP Barcode: D223738
ID#: 7E03489

SUBMISSION#: S446951
PC#:

Test Material: Benoxacor Technical (CAS#: [redacted]
Synonym: CGA-154281

Citation: Ian Munroe (August 9, 1993). The Significance of Forestomach Tumors Induced in Rodents by CGA154281. Prepared for Ciba Geigy and conducted by CANTOX Inc. of Canada for Ciba-Geigy, Greensboro, N.C., dated August 9, 1993. Unpublished (MRID# 428887-05).

Sponsor: Ciba-Geigy Ltd, Greensboro, NC

Action Requested: Review a report on the significance of forestomach tumors in rodents induced by CGA154281 (Benoxacor).

Background: This report was the result of a one-day symposium on issues of forestomach tumor formations in animals induced by CGA154281 (Benoxacor), organized by Ciba Geigy in May 1993. It was prepared for Ciba Geigy by Dr. Ian Munroe of CANTOX Inc of Canada. This document is a detailed analysis and review of all available data up to 1993 and included a comprehensive toxicological profile of CGA154281 (Benoxacor), based on studies that have been submitted to and reviewed by EPA as well citations from the open literature. The thrust of this report is to show that, based on the current understanding of forestomach tumorigenesis, the lesions induced by CGA154281 (Benoxacor) in animals are not relevant to humans exposed to this compound.

Discussion and Conclusion: Unless otherwise indicated the toxicological end points presented below are based on animal studies that have been reviewed by the Agency (HED's DER and Oneliners) or cited in the open literature:

A. Toxicological Profile based on Animal Data
   o Acute Toxicity

- The Oral LD_{50} is greater than 2000 mg/kg body weight in rats (Ciba Geigy, 1986)

- The Dermal LD_{50} is greater than 5000 mg/kg body weight in rats (Ciba Geigy, 1986)

- Dermal Penetration Study: After 10 hours, the total amount of chemical absorbed was 25.4% and 49.4% for the 1 and 10 mg/kg/day dose levels. After 24 hours, the total amount absorbed in treated animals was from 27.5 to 55.7%; 13 to 14% radioactivity was found in the dissolved skin after washing. The total ^{14}C recovery mean values for the 1 mg dosed rats at 2, 4 and 10 hours intervals ranged from 97.3 to 102.2%. (HED oneliners MRID407321-05).

o <u>Subchronic Toxicity</u>

- The systemic NOEL = 100 ppm (≈ 5 mg/kg/day) in rats; Systemic LOEL = 300 ppm (≈ 15 mg/kg/day) based on increased incidence of kidney nephrosis; Dosages: 0, 10, 100, 300, 1000 and 6000 ppm (≈ 0, 0.5, 5, 15, 20 and 300 mg/kg/day (HED oneliners; MRID#400288-12).

- The systemic toxicity NOEL = 500 ppm (70.7 and 99.8 mg/kg/day in males and females, respectively) in mice dosed for 3 months. The systemic toxicity LOEL = 2000 ppm (290 and 382 mg/kg/day in males and females, respectively), based on decreased body weight, renal cortex fibrosis and calcification in males, increased spleen weight in females, and increased water consumption, and liver and kidney weights in both males and females; doses: 0, 50, 500, 2000 and 6000 ppm (0, 7.14, 70.7, 290 and 1100 mg/kg/day for males and 0, 9.53, 99.8, 382 and 1470 mg/kg/day for females) (HED DER dated 5/16/1996 ; MRID#433374-03).

- The systemic NOEL = 5 mg/kg/day in dogs and the systemic LOEL = 50 mg/kg/day based on increased liver/gallbladder weight; doses: 0, 0.25, 1, 5, 50 and 150 mg/kg/day (HED oneliners; MRID#400288-12)

- The systemic NOEL is < 400 ppm (≈10 mg/kg/day) (only dose tested) in dogs based on increased liver/gallbladder weight and decreased RBC, HCT, HGB and total protein value; dose level tested is 400 ppm (≈10 mg/kg/day)(HED oneliners; MRID#400288-12).

o <u>Developmental and Reproductive Toxicity Studies</u>

- In a developmental toxicity study in rats dosed at 300, 600, 800 and 1000 mg/kg/day, the systemic maternal NOEL = 300 mg/kg/day and the maternal LOEL = 600 mg/kg/day based on increased abnormal clinical observations and decreased body weight gain. The developmental toxicity NOEL < 300 mg/kg/day (LDT) based on decreased fetal weight (HED Oneliners; MRID#400288-15).

- In a developmental toxicity study in rats dosed at 1, 100, and 400 mg/kg/day, the systemic maternal NOEL = 100 mg/kg/day and the maternal LOEL = 400 mg/kg/day based on increased maternal gross pathology findings, and decreased body weight gain. The developmental toxicity NOEL = 100 mg/kg/day and the developmental LOEL = 400 mg/kg/day based on decreased fetal weight, number of live fetuses, and decreased uterine weight, and increased early resorptions, fetal visceral variations, malformations and skeletal variations (HED Oneliners; MRID#400288-15).

- In a developmental toxicity study in rabbits dosed at 0, 0.5, 2.5, 12.5 and 62.5 mg/kg/day, the systemic maternal NOEL = 12.5 mg/kg/day and the maternal LOEL = 62.5 mg/kg/day based on decreased consumption values. The developmental toxicity NOEL = 12.5 mg/kg/day and the developmental toxicity LOEL = 62.5 mg/kg/day, based on increased frequency of vertebral anomaly with or without associated rib anomaly (Oneliners and DER/memo dated 12/9/88; MRID#400288-16).

- In a two-generation reproduction study, rats were fed in the diet with Benoxacor at doses of 0, 10, 50, 500 and 1000 ppm for two successive generations.
  The parental NOEL = 50 ppm (3.55 mg/kg/day in males and 4.51 mg/kg/day in females) and the parental LOEL = 500 ppm (34.84 mg/kg/day in males and 41.21 mg/kg/day in females), based on decreased body weight and body weight gain in both sexes and in both generations. The reproductive NOEL = 50 ppm (4.57 mg/kg/day) and the reproductive LOEL = 500 ppm (64.02 mg/kg/day for F1 and 73.25 mg/kg/day for F2 generations), based on decreased pup body weight on lactation day 21. (DER dated 9/8/95; MRID#428887-03)

o <u>Chronic and Oncogenicity Study</u>

- In a 12-month chronic oral study in dogs at 0, 1, 5, 40, 80 mg/kg/day, the systemic NOEL = 5 mg/kg/day and the LOEL = 40 mg/kg/day based on decreased mean body weight gain in males, increased liver and kidney weights and lipofuscin deposition in the kidneys, all noted in both sexes (HED DER dated 9/6/95; MRID# 428887-01).

- In a chronic study in mice, fed in the diet with Benoxacor for 80 months at 0, 10, 30, 600 and 1200 ppm (0, 1.2, 3.7, 75 and 167 mg/kg/day for males and 0, 1.6, 4.7, 93 and 201 mg/kg/day for females). The systemic NOEL = 30 ppm (3.7 and 4.7 mg/kg/day in males and females), respectively. The systemic LOEL = 600 ppm (75 and 93 mg/kg/day in males and females), respectively, based on increased incidence of forestomach excrescences, and increased liver/body weight ratio in both sexes (DER dated 9/15/95; MRID#428887-02).

- In a chronic/carcinogenicity study in rats, Benoxacor was fed in the diet for 2 years at 0, 10, 50, 500 and 1000 ppm

(0, 0.4, 2.0, 20.6 and 41.0 mg/kg/day for males and 0, 0.6, 2.8, 28.2 and 59.0 mg/kg/day for females). The systemic NOEL < 10 ppm (<0.4 and <0.6 mg/kg/day in males and females, respectively). The systemic LOEL = 10 ppm (0.4 and 0.6 mg/kg/day mg/kg/day in males and females, respectively), based on increased incidence of fatty hepatocytes in males, parafollicular cell hyperplasia in the thyroid and basophilic cortical tubules in the kidneys of males, and increased kidney congestion in the females (DER dated 9/15/95; MRID# 428887-04).

o <u>Mutagenicity Studies</u>

- **Ames Assay;** No evidence of mutagenicity to TA98, TA1537 and TA1538 strains of <u>Salmonella typhimurium</u> with or without metabolic activation; concentrations tested at 1000, 2000, 3000, 4000, 5000 and 8000 µg/ml in the first test and at 250, 500, 1000, 2000, 3000, and 4000 µg/ml in the second test (Oneliners; MRID#407321-03).

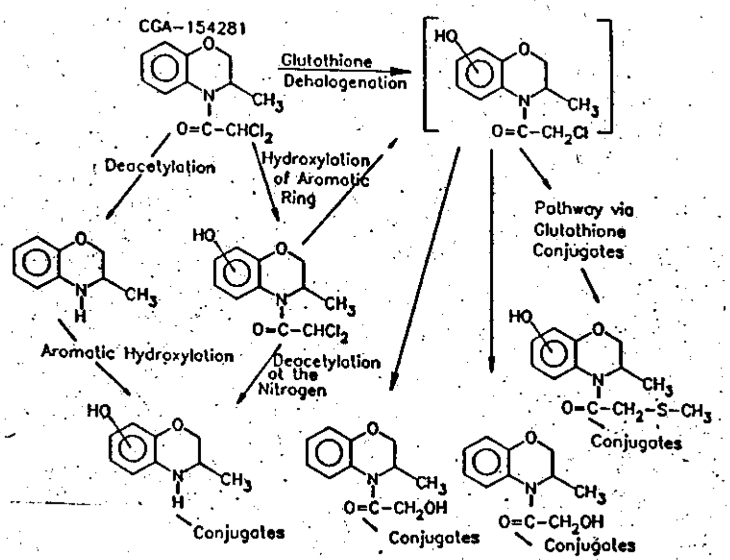
- **Unscheduled DNA Synthesis Assay:** Failed to cause DNA damage or inducible repair in the rat hepatocyte UDS assay at the following dose levels: 0.008, 0.04, 0.2, 1, 5, 10, 15, and 20 µg/ml, or 0.004, 0.002, 0.008, 0.04, 0.2, 1, 5, and 10 µg/ml (Oneliners; MRID#403888-12), or 0.1, 0.5, 2, 4, 6, 8, 10, and 20 µg/ml (Oneliners; MRID#403888-13).
  Benoxacor did not cause DNA damage nor induce repair in a human fibroblasts UDS assay without metabolic activation at concentrations of 0.25, 1.25, 6.25 and 31.25 µg/ml (Oneliners: MRID#403888-09).

- **Micronucleus Assay:** Dose range from 1250 to 5000 mg/kg. Under the condition of the study, no evidence of mutagenic effects was obtained in Chinese hamsters treated with Benoxacor Tech. There was no statistically significant increases in the number of micronucleated polychromatic erythrocytes as compared to the negative control animals; no clastogenic or aneugenic activity was reported (Memo Chen to Leifer dated 4/14/88; Oneliners; MRID#403888-08).

Based on the above mutagenicity studies, Benoxacor is considered non-genotoxic.

o <u>Metabolism</u>

The results of a metabolism study conducted by Cassidy and Kahrs (1986; MRID#4002288-22; DER dated 4/30/87), indicate that Benoxacor is rapidly absorbed, metabolized and excreted. The major route of elimination was via the urine (up to 70% of the dose recovered within 24 hours). Very little (<0.5% of the radiolabel), remained in the tissues of rats, 7 days after administration of either 0.5 or 500 mg/kg of Benoxacor. Based on the GC/MS analysis of the urinary metabolites, Benoxacor appears to be conjugated with glutathione, with subsequent dehalogenation, and also undergoes de-acetylation of the dichloroacetyl side chain to yield 3,4-dihydro-3-methyl-2H-1,4-benzoxazine; hydroxylation of the benzene ring also occurs. The proposed metabolic pathways are as follows:



o Hydrolysis of Benoxacor

It has been shown that Benoxacor is hydrolyzed very slowly in an acidic conditions (half-lives for hydrolysis was 922 days at Ph 5 and 670 days at Ph 3) and a slight amount of hydrolysis was noted in an alkaline conditions (half-lives for hydrolysis of 46-56 days at Ph 7 and 13-19 days at Ph 9) (Analytical Bio-Chemistry Lab., 1986; Agrisearch Inc., 1987). Because of these characteristics, therefore, it was postulated that it is unlikely that Benoxacor would undergo hydrolysis in the rodent acidic forestomach.

According to the author, based on the metabolic and hydrolytic findings, Benoxacor would be expected to be stable in the digestive tract and would not likely be de-acetylated through hydrolytic, non-enzymatic processes. Based on the proposed metabolic pathways shown above, the deacetylation product (the side-chain attached to heterocyclic nitrogen), possibly a dichloroacetic acid or chloroacetaldehyde, would have no contact with the forestomach tissues since the de-acetylation of the side-chain is likely to occur through enzymatic hydrolysis in the liver, not in the forestomach. Since no removal of the N-side chain is likely to occur in the forestomach, there is virtually no potential for the formation of a nitrosamine in the forestomach.

o <u>Tumor Incidences in the Forestomach in Rodents</u>

Tumors in the forestomach were noted in both the Rats and Mice long-term studies.

a. Incidence of Neoplastic and Non-Neoplastic Lesions in the Forestomach of Rats (MRID#428887-04) are summarized as follows:

Table 1: Rat Carcinogenicity Study

Forestomach Histopathological Findings	0 ppm	10 ppm	50 ppm	500 ppm	1000 ppm
	60♂/60♀	60♂/60♀	60♂/60♀	60♂/60♀	60♂/60♀
Excrescence (macroscopic)	1 / 1	0 / 1	0 / 0	6 / 3	12**/ 11**
Papillomatous hyperplasia	3 / 1	2 / 4	3 / 1	3 / 4	7* / 6*
Epithelial hyperplasia/hyperkeratosis	20 / 13	11 / 22	15 / 31	22 / 29	35**/ 31**
Squamous cell papilloma	0 / 1	0 / 0	0 / 1	0 / 1	4 / 4
Squamous cell carcinoma	0 / 0	0 / 0	0 / 0	0 / 0	0 / 1

* = P ≤0.05; ** = P ≤0.01; Derived from DER dated 9/15/95 of a Rat carcinogenicity Study; MRID#428887-04.

The above data show a treatment-related increased incidence of excrescences, epithelial and papillomatous hyperplasia in the forestomach. A slight increased incidence of Squamous cell papilloma was noted in the 1000 ppm males and females; these papillomas were predominantly located at the limiting ridge dividing the forestomach from the glandular stomach. No significant differences were note in the incidence of non-neoplastic lesions for any other organ sites.

Tissues of the forestomach of interim sacrificed and decedent rats that died during the first 52 weeks of treatment were re-evaluated histopathologically (DER dated 4/30/96;MRID#433374-06 (original submission MRID#428887-04). No treatment-related non-neoplastic lesions and no tumors were noted.

Histopathological re-evaluation of the stomach tissues in a subchronic feeding study in rats (DER dated 4/30/96; MRID#433374-05 (original Submission MRID#400288-12), revealed a statistically significant increased incidence of the non-glandular stomach hyperplasia in the 6000 ppm rats, and they are considered to be treatment-related. No stomach tumors were noted.

b. Incidence of Neoplastic and Non-Neoplastic Lesions in the Forestomach of Mice are summarized in Table 2:

Table 2: Mouse Carcinogenicity Study

Forestomach Histopathological Findings	0 ppm	10 ppm	30 ppm	600 ppm	1200 ppm
	50♂/50♀	50♂/50♀	50♂/50♀	50♂/50♀	50♂/50♀
Excrescence (macroscopic)	0 / 1	0 / 0	0 / 0	4 / 5	12**/ 15**
Papillomatous hyperplasia	0 / 0	0 / 0	0 / 0	0 / 1	10**/ 4*
Epithelial Hyperplasia	3 / 0	0 / 2	2 / 0	2 / 2	10*/ 3
Squamous cell papilloma	0 / 1	0 / 0	0 / 0	2 / 1	6 / 10**
Squamous cell carcinoma	0 / 0	1 / 0	0 / 0	1 / 1	3 / 1

* = P ≤0.05; ** = P ≤0.01; Derived from DER dated 9/15/95 of a Mouse Carcinogenicity Study; MRID#428887-02.

Table 2 shows statistically significant increased incidences of excrescences and papillomatous hyperplasia in the forestomach in the 1200 ppm male and female mice. The incidence of excrescence in the forestomach was also increased in the 600 ppm mice, but these increases were not statistically significant. A statistically significant increased incidence of Epithelial hyperplasia in the males and squamous cell papilloma in the females was noted in the 1200 ppm dose groups. These papillomas were predominantly located at the limiting ridge dividing the forestomach from the glandular stomach. A slight increase incidence of squamous cell papilloma was found in the 1000 ppm rats, indicating a possible weak carcinogenic response. No significant differences were noted in the incidence of non-neoplastic lesions for any other organ site.

Re-evaluation of histo-pathological findings of the forestomach in a 90-day feeding study in mice are summarized in Table 3.

Table 3

Forestomach Histopathological Findings	0 ppm	50 ppm	500 ppm	2000 ppm	6000 ppm
	10♂/10♀	10♂/10♀	10♂/10♀	10♂/10♀	10♂/10♀
Nonglandular Stomach -Epidermal Cyst -Inflammatory Cell Infiltration -Lymphocytic Infiltration -Epithelium Hyperplasia	 0 / 1 5 / 3 0 / 0 1 / nr	 0 / 0 2 / 1 1 / 1 0 / nr	 1 / 0 2 / 2 0 / 0 0 / nr	 0 / 0 1 / 1 0 / 0 1 / nr	 0 / 0 1 / 1 0 / 0 0 / nr
Glandular Stomach -Inflammatory Cell Infiltration -Lymphocytic Infiltration	 1 / 1 0 / 0	 2 / 3 0 / 1	 3 / 4 0 / 0	 0 / 1 1 / 0	 1 / 2 0 / 0
Gastric Mucosa -Erosion -Necrosis -Ulceration -Hyperplasia	 0 / 1 1 / nr nr/ 1 nr/ 1	 1 / 1 0 / nr nr/ 1 nr/ 0	 1 / 1 0 / nr nr/ 1 nr/ 0	 1 / 0 1 / nr nr/ 0 nr/ 0	 0 / 0 0 / nr nr/ 0 nr/ 0
Gastric Gland Dilatation	5 / 2	0 / 2	2 / 1	4 / 1	1 / 2

nr = not reported; derived from a 90-day feeding study in mice (DER dated May,1996; MRID#433374-03).

The above data did not show any treatment-related changes in either the glandular or nonglandular stomach in mice.

- Current Understanding of Forestomach Tumorigenesis

The mechanisms of chemicals inducing carcinogenesis are complex and are not fully understood. According to the author, it is currently generally accepted that two general mechanisms by which chemicals may induce carcinogenesis:

1. One mechanism is characterized by the interaction of the chemical or its metabolites with nucleic acids resulting in mutations which can ultimately lead to the induction of self-replicating lesions as cell divisions and growth. These are chemicals which are capable of interacting with DNA and which show positive results in short-term in vitro and in vivo mutagenicity tests.

2. The second is represented by chemicals that may induce carcinogenesis through a threshold-dependent, epigenetic, toxic effect. Carcinogenesis induced by non-mutagenic chemicals often involves the induction of cell proliferation as a result of high-dose toxicity. With increased cell proliferation (hyperplasia), this may promote the replication of spontaneously initiated cells or provide additional opportunities for spontaneous mutations in DNA to occur. Chemicals acting via a non-genotoxic epigenetic mechanism, are not likely to induce carcinogenesis at doses below which it does not induce the toxic effects associated with cell proliferation.

The dose-response relationships, tumor development sequences, lesion reversibility, relationship to cell proliferation, and tumor induction at sites other than the forestomach by other chemicals [such as butylated hydroxyanisole (BHA), propionic acid (PA) and ethyl acrylate (EA)], which induce forestomach tumors through non-genotoxic mechanisms, are discussed below.

o Dose Response Relationship

Non-Mutagenic Forestomach Carcinogen
The dose-response for the induction of tumors by non-mutagenic carcinogens tends to be highly non-linear and usually shows a clear threshold for the induction of tumor response as shown by the following example:

- Butylated hydroxyanisole (BHA)
BHA has been reported to induce forestomach tumors in rats, mice and hamsters, showing threshold characteristics of a non-mutagenic carcinogens. A clear threshold for the induction of papillomas in rats was noted at doses in excess of 0.5% of BHA in the diet. At a 0.5% BHA dose level,

papillomas were found in 2% of the male and female rats; at 1.0% of a BHA dose level, papillomas were found in 75.5% of the male rats;at a 2% BHA dose level, papillomas and squamous cell papillomas were found in 91.5% and 13.8% of male rats, respectively; at a 2.0% nominal concentration dose level, papillomas were noted in 100% of the male and in 96.1% of the female rats, and squamous cell papillomas were observed in 13.8% of the male and 29.4% of the female rats, respectively (Ito et al, 1985 and Masui et al, 1986).

- Propionic Acid

  Rats fed with propionic acid in the diet at 4000 and 40000 ppm showed a similar pattern; at a 40000 ppm dose level, extensive hyperplasia, ulceration, papillomatous growth and "carcinomatous changes" of the forestomach epithelium were noted; at 4000 ppm dose level, only hyperplasia of the forestomach epithelium was seen and no tumors were noted (von Greim,1985).

- Ethyl Acrylate (EA)

  In a NTP (1983) study, Ethyl acrylate administered by gavage to mice and rats for 2 years at doses of 100 and 200 mg/kg/day induced hyperproliferative forestomach tumors in both species as summarized in Table 3.

Table 3

Species	Control		100 mg/kg/day		200 mg/kg/day	
	Papilloma (%) ♂ / ♀	Squamous Cell Carcinoma (%) ♂ / ♀	Papilloma (%) ♂ / ♀	Squamous Cell Carcinoma (%) ♂ / ♀	Papilloma (%) ♂ / ♀	Squamous Cell Carcinoma (%) ♂ / ♀
Rat	2.0 / 2.0	0 / 0	30.0 / 12.0	10.0 / 0	58.0 / 18.0	24.0 / 4.0
Mouse	0 / 2.0	0 / 0	9.0 / 8.0	4.0 / 2.0	18.0 / 10.0	10.0 / 4.0

Derived from p.18 of Ian Munro Report as cited from the 1983 NTP Report (August 9,1996; MRID#428887-04).

Increased incidence of squamous cell carcinoma was noted primarily in the 200 mg/kg/day male mice and rats. The NTP study design at these high dose levels precludes an evaluation of the dose-response curve. However, other evidence such as the association of neoplastic lesions with induction of cell proliferation, the time progression of lesion formation, the reversibility of antecedent non-neoplastic lesions, and the lack of induction of tumors at any other site provides strong support for the consideration of ethyl acrylate as a threshold-dependent carcinogen.

The above are typical examples for non-mutagenic compounds which induce carcinogenesis as a result of tissue toxicity, irritation, and/or concomitant cell proliferation. The forestomach tumor dose-responses for BHA and propionic acid are all highly non-linear in nature, and show a clear threshold for tumor induction.

Genotoxic Forestomach Carcinogen

Genotoxic forestomach carcinogens induces tumors at very low doses; several agents are capable of inducing non-neoplastic lesions and/or tumors as a result of a single, or few, oral doses.

N-methyl-N-nitro-Nitrosoguanidine (MNNG)

- N-methyl-N-nitro-nitrosoguanidine (MNNG) is a classical example of a genotoxic forestomach carcinogen, inducing tumors as a result of considerably less than lifetime exposure in animals. It was reported that single oral doses of 50 to 250 mg MNNG/kg induce forestomach tumors in rats (Craddock, 1968; Hirono and Shibuya, 1972). No non-genotoxic forestomach carcinogen is known to induce tumors as a result of a single oral dose.
- Benzo(a)Pyrene [B(a)P]
  Administration of B(a)P in the diet for 10 to 22 weeks in mice induced increased forestomach tumors in a dose-related manner, with incidences as high as 90% in 250 ppm dosed mice. Mice fed 250 ppm B(a)P in the diet for only 4 days, developed forestomach tumors 155 days post-dosing (Neal & Rigdon, 1967). The fact that tumor effects were induced as a result of only 4 days of feeding is indicative of the strong tumor initiator potential of B(a)P.
- Dibutylnitrosamine (DBNA)
  Mice fed with 50 ppm DBNA in the diet for 52 weeks, induced forestomach papillomas and squamous cell carcinomas in all 33 animals (Takayama and Imaizumi, 1969).
  DBNA at a dose of 44 mg/kg/day via gavage in hamsters induced forestomach carcinomas in 32% and papillomas in 59% of the animals (Althoff et al, 1973).
- 1,2-Dibromoethane
  1,2-dibromoethane is mutagenic in most short-term tests and has been reported to induce forestomach carcinomas in mice and rats in chronic studies. Mice dosed intermittently via gavage at 62 and 107 mg/kg/day for 53 weeks, followed by observation until 78 weeks, produced forestomach squamous cell carcinoma in 55% and up to 93%, respectively. Rats dosed intermittently via gavage at 37 and 41 mg/kg/day for a total of 34 and 57 weeks, respectively, produced forestomach squamous cell carcinoma in 58% and 90%, respectively (NCI, 1978)

Based on the above examples, it can be concluded that non-mutagenic forestomach carcinogens tend to induce tumors in a threshold-dependent, non-linear fashion, at higher doses and longer dosing periods as compared to mutagenic forestomach carcinogens. Non-mutagenic forestomach carcinogens induce relatively less malignant tumors, especially at non-toxic doses, as compared to mutagenic carcinogens. It should be noted that almost all of the forestomach tumors induced by 1,2-dibromoethane in tested animals were malignant.

o Progression of Tumor Development and Reversibility of Lesions

The progression of neoplastic lesions induced by non-genetic carcinogens starts with cellular proliferation and hyperplasia in response to the toxic insult followed by the development of non-malignant papillomatous or adenomatous tumors after continued stimulation of the hyperplastic response. Continued long term exposure at high doses of cytotoxic/irritant chemicals will eventually lead to the promotion of adenomatous tumors or hyperplastic nodules to a malignant state. Cessation of exposure to non-genotoxic carcinogens generally leads to a regression of cell proliferation and hyperplasia as well as regression of most papillomas and adenomatous tumors, without any further progression to a malignant state; a typical example of this mechanism is the case of an antioxidant compound BHA (Butterworth, 1990). Sequential development of forestomach lesions were also noted in studies with ethyl acrylate; severe mucosal inflammation and edema in the forestomach were noted in rats after gavage administration of ethyl acrylate at 200 or 400 mg/kg; creamy white mucosal nodules formed in the fore-stomach with microscopic evidence of hyperplasia and hyper-keratosis following a 2-week dosing at 100 and 200 mg/kg; after 13-week dosing forestomach epithelial thickening was noted in both doses, but the focal and multifocal nodular proliferation was only noted in the 200 mg/kg dose group. The late development malignancy, development of lesions through a time-dependent sequence involving hyperplasia, cell proliferation, and papilloma formation, an eventually the occurrence of neoplasia after a long pre-malignant stage is typical for non-genotoxic carcinogens (Clayson 1989; Clayson et al, 1989; Butterworth, 1990).0

Genotoxic forestomach carcinogen [e.g Benzo(a)pyrene] produced malignant lesions in a shorter timeframe as compared to non-genotoxic carcinogens. In feeding studies in mice with benzo(a)pyrene, squamous cell carcinomas were noted after 14 to 17 weeks of dosing at 100 ppm, and in 16 to 29 weeks with a 50 ppm dose level.

Another genotoxic carcinogen, 1,2-dibromoethane, induced forestomach tumors, almost all of which were squamous cell carcinomas, in 12 weeks in rats dosed via gavage at 37 to 41 mg/kg/day, and in mice within 24 weeks dosed at 62 to 107 mg/kg/day (NCI, 1978).

Based on the above evidence, non-genotoxic carcinogens, clearly indicate lesion development that is dose- and time-dependent with a recognizable sequence of lesion progression involving initial inflammation, followed by cell proliferation and hyperplasia, nodule formation (papillomatous hyperplasia), then followed by the appearance of papilloma, and finally carcinoma. Cessation of dosing prior to the development of overt tumors usually results in the regression of the preneoplastic lesions. In contrast, genotoxic forestomach carcinogens, induce carcinoma in a very short time period without the intermediate formation of antecedent lesions; generally, lesions induced by genotoxic carcinogens are not reversible after cessation of exposure.

- Cell Proliferation Induced by Chemicals

  Inflammatory responses, tissue necrosis, hyperkeratosis, hyperplasia and dysplasia were noted preceding forestomach tumor formation in rats exposed to BHA at high doses in the diet (Clayson, et al, 1986; Masui et al, 1986a; Altman et al, 1986). Increased incidence of papillomatous hyperplasia was noted in 1200 ppm animals, with the increase being of statistical significance in males. Concomitant with hyperplasia, excrescence(s), and limiting ridge thickening was an increase in the incidence of squamous cell papilloma. The appearance of excrescence, hyperplasia and limiting ridge thickening is indicative of a persistent irritant and/or toxic effect of benoxacor on the forestomach epithelium. The strong supporting evidence that the papillomas induced by benoxacor is due to a cytotoxic effect, is based on the concomitant appearance of papillomas and excrescence(s) in rats and mice, with many of these mice also exhibiting epithelial hyperplasia, papillomatous hyperplasia, and hyperkeratosis.

- Tumor Response at Other Sites

  Benoxacor induces a single organ tumor response, that is the forestomach, most of which are benign, in rodents exposed through the diet. This suggests that the mechanism of action of benoxacor may be similar to that of non-genotoxic forestomach carcinogens (i.e. cell proliferation induced by forestomach epithelial irritation and toxicity).

- Significance to Humans of Forestomach Tumors Induced by Benoxacor in Rodents

  Given that benoxacor produces tumors in rodent forestomach, the mode of action of tumor induction associated with tissue irritation, and that no tumors are expected at doses below which no toxic effects on the forestomach epithelium are noted in rats, the likelihood of tumor formation induced by benoxacor in humans is minimal, because humans have no forestomach and human exposure to benoxacor is well below that experienced by rats. Thus, the forestomach tumors seen in experimental animals may be of no relevance to humans on a mechanistic basis alone.

  Based on the results of the three chronic studies, rats, mice and dogs, the lowest NOEL is 0.4 mg/kg body weight/day based on reduced body weight gain and mild hepatoxicity noted in male rats in a chronic/carcinogenicity study (Ryle et al, 1993).

Human exposure to Benoxacor was estimated to be about 0.000187 mg/kg/day for the U.S. population as a whole and up to 0.000888 mg/kg/day for non-nursing infants (highest of any population subgroup). These exposure estimate assumed a 0.01 ppm residue for all metolachlor-treated commodities, and a 100% market share for these products.
Based on an assumed human exposure of 0.000187 to 0.000888 mg/kg/day and a NOEL of 0.4 mg/kg/day, any human exposures to Benoxacor will be far below (450- to 2,139-fold) the NOEL for forestomach tumors determined in experimental animal studies.

CONCLUSIONS

Based on the discussions and data presented above, the following major conclusions were made by the author:

- The dose response of non-genotoxic carcinogens tends to be non-linear and to show a threshold for tumor induction.

- The progression of lesions induced by non-genotoxic carcinogens shows a precise sequence, induction of hyperplasia, followed by the development of papillomas and finally carcinomas. Latency period for induction of tumors covers a major part of animal's lifetime. Lesions tend to be reversible prior to tumor formation.

- Non-genotoxic forestomach carcinogens induce tumors that are clearly related to antecedent or concomitant induction of cell proliferation and hyperplasia. Genotoxic forestomach carcinogens, do not necessarily induce cell proliferation and are capable of inducing forestomach tumors without inducing a cytotoxic or irritant response in the forestomach epithelium.

- Non-genotoxic forestomach carcinogens only affect this organ, while genotoxic forestomach carcinogens are usually active at many other organ sites indicative of the systematic mode of action of these compounds.

Based on the weight of evidence presented above, the characteristics of the Benoxacor forestomach tumor response are consistent with a nongenotoxic mechanism of action. Benoxacor is a non-genotoxic compound which induces a threshold-dependent, weak tumorigenic effect on the forestomach of rats and mice treated orally with Benoxacor. The forestomach tumors are associated with repeated tissue irritation or cytoxicity and hyperproliferation and do not arise <u>de novo</u>. From the above discussions, on the whole, the tumor responses of butylated hydroxyanisole and benoxacor are similar when considered on a per dose basis.

Human exposure to Benoxacor was estimated to be about 0.000187 mg/kg/day for the U.S. population as a whole and up to 0.000888 mg/kg/day for non-nursing infants (highest of any population

subgroup). These exposure estimates assumed a 0.01 ppm residue for all metolachlor-treated commodities, and a 100% market share for these products.

Based on an assumed human exposure of 0.000187 to 0.000888 mg/kg/day and a NOEL of 0.4 mg/kg/day, any human exposures to Benoxacor will be far below (450- to 2,139-fold) the NOEL for forestomach tumors determined in experimental animal studies.

In conclusion, the forestomach tumors induced by benoxacor noted in rodents may not be relevant to humans based on both the mode of mechanism (as shown through comparison to other non-genotoxic and genotoxic forestomach carcinogens) and based on the potential human exposure under the present use pattern.

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